infections possibly hit during a damp winter during which other microbes are never far away and some are already present in the lungs. The human immune system has certainly evolved under strong pressure to integrate past and simultaneous microbial encounters, to avoid overshooting as well as insufficient or inappropriate responses. Schliehe *et al.* delineate a novel molecular mechanism by which an ongoing immune response affects a subsequent one[1]. More studies like this will be needed to figure out how this sometimes undesired interference works, not least to find better prophylactic and therapeutic interventions to stop the bacterial executioners in severe coinfection.

COMPETING FINANCIAL INTERESTS
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# A surprising role for TLR7

**Michael M Lederman**

**Ligation of the Toll-like receptor TLR7 in human CD4$^+$ T cells elicits an anergic state that may contribute to CD4$^+$ T cell hyporesponsiveness after infection with human immunodeficiency virus type 1 and may also enhance propagation of this virus.**



TLR7 is one of several Toll-like receptors (TLR3, TLR7, TLR8 and TLR9) that recognize microbial nucleic acid sequences. TLR7 and TLR8, which recognize single-stranded RNA, are distributed broadly among myeloid and other cells and are characteristically expressed in endosomal compartments, where their engagement with microbial sequences is thought to take place. In this issue of *Nature Immunology*, Dominguez-Villar *et al.* demonstrate a surprising role for TLR7 in host-virus relationships[1]. In a paper fraught with novelty, the authors provide evidence that engagement of TLR7 expressed in primary human CD4$^+$ T cells can induce anergy. They also find that activation of a TLR7-dependent mechanism in CD4$^+$ T cells promotes the replication of human immunodeficiency virus type 1 (HIV-1) within these cells—a hitherto unexpected role for this microbial sensor.

The authors first report that incubation of human CD4$^+$ T cells with various TLR7 agonists such as imiquimod impairs their proliferation and the expression of various cytokines in response to costimulation via the invariant signaling protein CD3 and coreceptor CD28. They do not obtain a similar effect with TLR8 agonists, and this effect of TLR7 stimulation is abrogated by silencing of TLR7-encoding RNA; therefore, the proanergic effect seems to be a specific property of TLR7 ligation. There is an emerging literature suggesting that non-synchronized activation of the T cell antigen receptor and costimulation results in calcium flux and induction of anergy through activation of the transcription factor NFAT1 (ref. 2). Dominguez-Villar *et al.* now show that engagement of TLR7 in CD4$^+$ T cells results in induction of calcium flux and activation of the transcription factor NFATc2 via dephosphorylation that then drives the expression of a host of anergy-related genes[1]. This effect is abrogated by silencing of the gene encoding NFATc2. The engagement of TLR7 with imiquimod also results in phosphorylation of the transcription factor NF-κB and the signaling molecules IRAK4 and p38 but lower basal expression of the signaling molecule Jnk and less phosphorylation of Jnk and its target Jun (a component of the transcription factor AP-1) induced by the phorbol ester PMA plus ionomycin. Collectively these results offer two potential means by which stimulation via TLR7 diminishes the immunological responsiveness of CD4$^+$ T cells: one by the induction of various anergy-related genes, and the other by interference with AP-1-dependent signals.

To try to place their findings into a more physiological context, the authors seek to understand whether the hyporesponsive state of CD4$^+$ T cells seen in HIV infection could be explained by that TLR7 effect. The authors confirm earlier reports that infection of CD4$^+$ T cells with HIV-1 *in vitro* diminishes their ability to produce the cytokines IL-2 and IFN-γ after restimulation. Productive infection is apparently not necessary for this anergic effect, as cells that have not supported expression of an HIV-1 reporter gene seem to be as compromised in these assays as are their neighboring productively infected cells. It might be a stretch to contend that this phenomenon drives the dysfunction of CD4$^+$ T cells in untreated HIV infection, as an even smaller minority of CD4$^+$ T cells seem to be HIV-1 infected *in vivo*. However, in the setting of unrestrained viral infection that is linked to both clinical evidence and laboratory evidence of CD4$^+$ T cell dysfunction, it may be possible that many CD4$^+$ T cells are in fact exposed to virus, viral proteins or viral sequences that do not reflect completion of the viral life cycle but still may engage the coreceptor CD4, TLR7 or other innate sensors[3].

The authors then report an unexpected role for engagement of TLR7 that facilitates the infection of CD4$^+$ T cells with HIV-1, as silencing of the gene encoding TLR7 or blockade of this innate receptor with the inhibitor IRS661 attenuates infection. Here too a role for NFATc2 is suggested, as silencing of the gene encoding NFATc2 or blockade of NFATc2 itself also attenuates HIV-1 replication. The authors conclude that HIV-1 uses the anergic state induced by ligation of TLR7 to support its own propagation. As best as can be seen, their systems use broad activation of T cells to render target cells susceptible to *in vitro* infection, but still it is fascinating to propose that HIV has 'learned' to utilize for its own propagation in CD4$^+$ T cells an innate sensor that is typically used by dendritic cells, monocytes and

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macrophages to arm host defenses against viruses. Indeed, the authors find no evidence that engagement of TLR7 on CD14$^+$ monocytes leads to a hyporesponsive state; instead, and as expected, these cells are activated following exposure to imiquimod. Ligation of TLR7 in CD4$^+$ T cells therefore seems to trigger a response entirely different from that seen in monocytes. Why would this be? At this point, researchers can only speculate. Perhaps engagement of TLR7 helps to defend (in myeloid cells, via the activation of antiviral defenses; and in T cells, via the attenuation of their activation) against archaic retroviruses that have successfully populated 7% of the human genome. Moreover, while the activation of T cells drives adaptive immunity, it seems that checks and balances are needed, such that signals are coordinated in place and time (and magnitude) to promote an effective (and tolerated) immune response.

On the other hand, the authors propose that this model of TLR7-dependent anergy has a role in the persistence of HIV and at the level of sustaining virus-infected cells and impairment of adaptive defenses, this may be so, but there are aspects of this story that will need some more sorting out. For example, does the engagement of TLR7 take place after viral entry and 'decapsidation', or do newly transcribed viral RNAs bind TLR7, either way sustaining the anergic state and promoting viral replication (**Fig. 1**)? Do viral sequences enter TLR7-containing endosomes via autophagy[4], or do some viruses enter CD4$^+$ T cells via endosomes[5]? And at what stage of the viral life cycle does this anergic state enhance viral replication? The methods used here do not distinguish events before integration from those after integration, but the activation of both NFATc2 and NF-κB by ligation of TLR7 is compatible with the idea that engaging TLR7 in CD4$^+$ T cells may enhance transcription from the viral promoter.

However, in myeloid cells, ligation of TLR7 typically initiates an antiviral state by driving expression of type 1 interferons that arm global antiviral defenses and also induce expression of various elements that restrict HIV replication directly[6]. In this context, it remains unknown whether TLR7 ligation or HIV replication within these CD4$^+$ T cells activates a type I interferon response and whether the immunosuppressive effects of type I interferons acting in *trans* contribute to the broad impairment of immunological function seen here even in cells that are not HIV-1 infected.

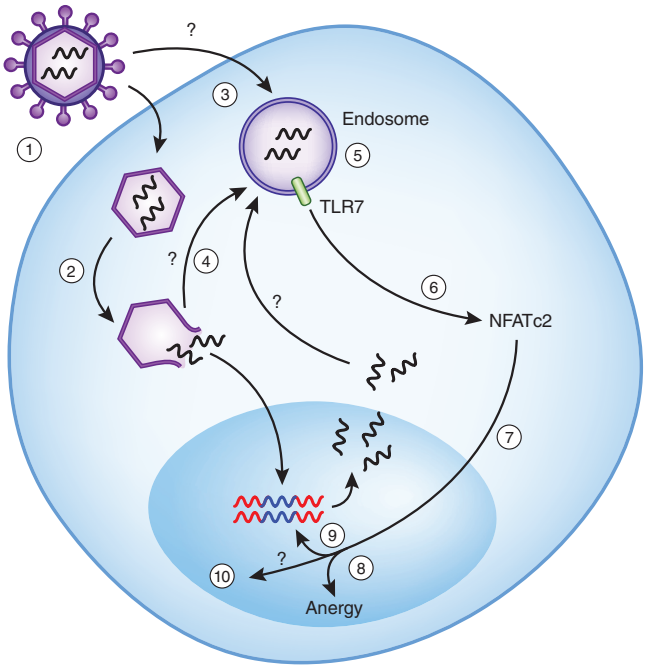


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**Figure 1** The engagement of TLR7 in isolated CD4$^+$ T cells induces an anergic state and promotes HIV-1 replication. HIV enters CD4$^+$ T lymphocytes after fusion with the cell membrane (1). As the capsid decomposes, viral RNA is reverse-transcribed and proviral DNA then enters the nucleus and integrates into the host genome (2). Potentially, some virions could enter the cell via endosomes (3); alternatively, during decapsidation, some virions might enter phagosomes via autophagy (4). Within endosomes, TLR7 is engaged by viral single-stranded RNA, which results in calcium flux (5). The calcium flux results in dephosphorylation of NFATc2 (6), which translocates to the nucleus (7), where a series of anergy-related genes are induced (8). Activated NFATc2 (and NF-κB that is activated by ligation of TLR7) may also enhance the transcription of viral RNA from integrated HIV (9) or could potentially induce expression of other host factors that promote the HIV replication cycle at sites to be determined (10).

Is TLR7-induced anergy a prerequisite for HIV-1 infection? Perhaps in this system it is, but it may be overreaching to conclude that this phenomenon is central to HIV propagation, as both here and in other laboratory settings, activation of CD4$^+$ T cells is used to render cells more readily infectible, and in clinical settings, activation of the immune system is linked to HIV replication both as its consequence and also as its plausible driver[7]. However, maybe there is some warning to 'lumpers' (like me) that the relationship between immunity and viral propagation in HIV infection might be more nuanced. Indeed, current fashion dictates that HIV 'likes' activation of the immune system, replicating better in activated cells than in resting cells, and somehow drives activation of the immune system, pathogenesis and morbidity. However, at the same time, people with HIV infection can have a profoundly impaired immune system, with not only decreased numbers of circulating CD4$^+$ T cells but also a functional anergy-like impairment of the cells that remain. On the other hand, it is possible that the systems applied to test models *in vitro* introduce more paths and diversions than are justified by whole-person biology. Nonetheless, this is a lovely, clearly presented work that will undoubtedly stimulate more of the same, plenty of arguments and maybe even the hurling of some fruit. Hurrah!

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The author declares no competing financial interests.

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